Correlations between arsenolipids, organic and inorganic forms of arsenic, mercury and selenium in muscles and cephalothoraxes of Aristaeomorpha foliaceashrimp

Soultani, G.; Sele, Veronika; Rasmussen, Rie Romme; Pasias, I.; Stathopoulou, E.; Thomaidis, Nikolaos S.; Scoullos, M.; Sloth, Jens Jørgen

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

Correlations between arsenolipids, organic and inorganic forms of arsenic, mercury and selenium in muscles and cephalothoraxes of *Aristaeomorpha foliacea* shrimp.

Hellenic Republic National and Kapodistrian University of Athens

National Food Institute DTU Technical University of Denmark

G. Soultani[1,3]*, V. Sele[2], R.R. Rasmussen[3] , I. Pasias[1], E. Stathopoulou[1], N. Thomaidis[1], M. Scoullos[1] and J. J. Sloth[2,3]

[1]National and Kapodistrian University of Athens (NKUA), Panepistimioupolis Zographou, 15771 Athens, Greece
[2]National Institute of Nutrition and Seafood Research (NIFES), N-5817 Bergen, Norway
[3]National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK 2860 Søborg, Denmark
*Corresponding author: E-mail: gesoultan@chem.uoa.gr

Introduction

A Mediterranean species of shrimps, *Aristaeomorpha foliacea* (giant red shrimp) from the deep waters of the Ionian Sea, was studied in terms of its content of organic and inorganic arsenic (As), mercury (Hg) and selenium (Se) in the muscles and cephalothoraxes (head including hepatopancreas). Generally, total As concentration ranges from 1 to 100 mg/kg w.w. in marine organisms. As is found in marine organisms as inorganic (the most toxic form of As, typically found in low concentrations) and as organic (considered as less- or non-toxic, and typically found in high levels). Arsenolipids, a group of lipophilic of arsenic-containing compounds, has been reported in concentrations from 1 to 50 mg/kg in marine oils [1]. Hg, a well-known toxic element, is typically determined in low levels in crustaceans. Se is believed to have an antagonistic protective effect against the toxicity of Hg and hence the ratio of these two elements is interesting to be studied in marine organisms.

Aristaeomorpha foliacea (giant red shrimp) from Killini (Ionian Sea)

Aim

The aim of the present study was to determine the levels of the total As and its forms (organic and inorganic As), as well as the levels of Hg and Se in order to evaluate the food safety of this type of shrimp.

Materials and Methods

- **Total Arsenic and Selenium**

Digestion in a microwave system by the use of acids (HNO_3 and H_2O_2)
Final determination by Inductively Coupled Plasma Mass Spectrometry (ICP-MS 7700x, Agilent Technologies)
Instrumental conditions: RF power: 1550 W; Plasma gas flow: 15 L/min; Carrier gas flow: 1.0 L/min; Make-up gas flow: 4.2 L/min; Torch: 2.5 mm i.d.; Nebulizer: OpalMist; Integration time:1000 ms

- **Total Arsenic of fat oil**

Extraction of the fat oil containing the arsenolipids by the Bligh and Dyer method [2]
Digestion in a microwave system by the use of acids (HNO_3 and H_2O_2)
Final determination by ICP-MS (ICP-MS 7500ce, Agilent Technologies)
Instrumental conditions: RF power: 1500 W; Plasma gas flow: 15 L/min; Carrier gas flow: 0.9 L/min; Make-up gas flow: 0.16 L/min; Torch: 2.5 mm i.d.; Nebulizer: Babington; Integration time:1000 ms

- **Inorganic Arsenic**

Extraction in waterbath with 0.1M HNO_3/ 3% H_2O_2.
Final determination by High Performance Liquid Chromatography coupled to ICP-MS (HPLC 1260, / ICP-MS 7500ce, both Agilent Technologies)
Instrumental conditions: Column: Anion Exchange (Trangenomic, 120*4.6mm); Method: Isocratic elution; Injection Vol.: 25 µl; Flow: 1 mL/min.; Column Temperature: 30°C; Mobile phase: 50 mM $(NH_4)_2CO_3$ in 3% MeOH (10 min.)

- **Organic Arsenic**

Was calculated by the substraction of inorganic arsenic from total arsenic

- **Arsenolipids**

Extraction of the fat oil containing the arsenolipids by using the Bligh and Dyer method
1.0 g of oil was partitioned between n-heptane (7.5 mL) and MeOH/H_20 (9:1 v/v; 2*3.75mL). The MeOH phases were evaporated to dryness and dissolved in 0.5 mL MeOH/H_20 and filtered. The samples further diluted in MeOH/H_2O prior the analysis
Determination by HPLC-ICPMS [3] (HPLC 1260 / ICP-MS 7500ce, both Agilent Technologies)
Instrumental conditions: i) HPLC column: Waters Acquity BEH C18 (2.1*100 mm, 1.7 µm); Mobile phases: A) 0.1% formic acid in H_20, B: 0.1% formic acid in MeOH; Gradient Program: 0-2 min: 30-100% B, 2-32 min:100% B, 33-45 min:30% B; Flow: 0.14 mL/min; Injection Vol.: 3 µL; Column Temperature: 30°C; ii) ICP-MS: RF power: 1530 W; Plasma gas flow: 15 L/min; Carrier gas flow: 0.22 L/min; Make-up gas flow: 0.46 L/min; Torch: 1.5 mm i.d.; Nebulizer: Micromist, 10-100 µL/min; Integration time:100 ms

- **Mercury**

Digestion in a microwave system by the use of acids and final determination by Cold Vapour Atomic Fluorescence Spectrometry (CVAFS) purge and trap dual amalgamation thermal extraction manual system coupled with Tekran detector, according to the EPA method 1631

Results & Conclusion

The total As in the muscles was 16.3 mg/kg w.w., while in the cephalothoraxes much higher concentrations (32.7 mg/kg w.w.) were observed. The inorganic form of As in the shrimp was detected only in the cephalothoraxes at low levels (0.9 µg/kg w.w.) and not at all in the edible muscle tissue. Arsenolipids comprised 0.4% of the total organic As in the muscles and 1.9% of the organic As in cephalothoraxes. Analysis of the cephalothorax extract showed the existence of several arsenolipids and it was compared with the arsenolipid known profile of commercial oil as shown to the **Figures 1 and 2** below. The chemical structures of the identified arsenic-containing peaks of the commercial oil were: i) arsenic –containing hydrocarbons (AsHC-R, R=C_{15},C_{17},C_{21}) and ii) arsenic-containing fatty acids (AsFA-R, R=C_{21}, C_{22}) **Figure 3**.The rest of the organic As, including the water-soluble compounds, mainly arsenobetaine, which typically is the predominant arsenic compound in marine organisms, was calculated. As far as Hg is concerned according to the European Legislation for heavy metals in foods, it has a maximum level of 0.5 mg/Kg w.w. in seafood. The studied samples had a lower concentration than the maximum level. The concentration of Se in the whole shrimp (sum of both tissues) was 3 times higher than the Hg concentration. Remarkably, Se content in cephalothoraxes was one folder higher. The correlation coefficient between these two metals had the highest value (-1.00). In conclusion, even though the levels of As were quite high, the largest proportion of total As was in organic form (primarily non-toxic) and the levels of Hg were lower than the permissible maximum level. The cephalothorax oil analyzed contained 7-8 arsenolipid compounds. However, it is not clear if each peak corresponds to a single compound, or several compounds of similar character, so further work will be needed.

	total As mg/kg w.w.	inorganic As mg/kg w. w.	organic As mg/kg w. w.	total As mg/kg in oil	total Arsenolipids mg/kg w. w.
Muscles	16.30	<0.0002	16.30	8.310	0.0615
Cephal.	32.70	0.0009	32.70	7.890	0.6170

Table 1: Content of total arsenic and its forms

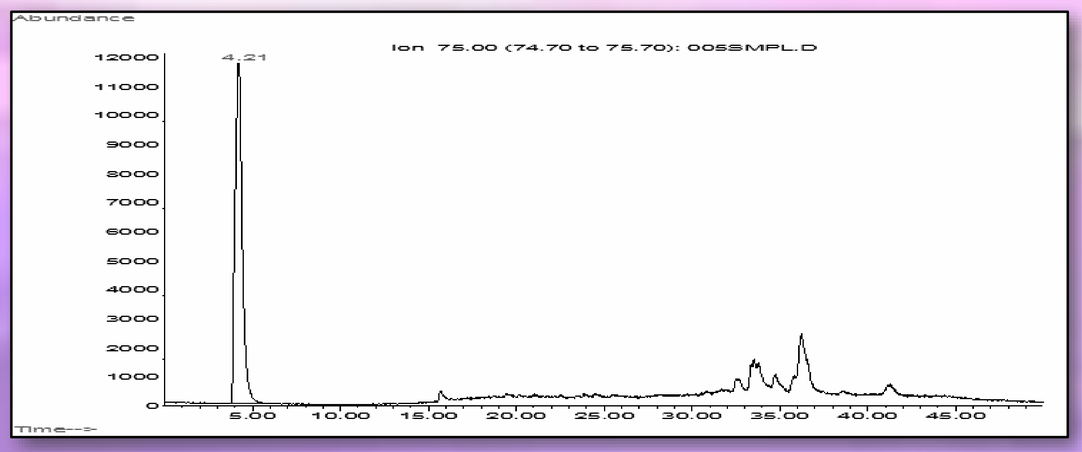

Figure 1: Arsenolipids; chromatogram of the aqueous methanol phase of oil from cephalothoraxes analyzed by HPLC-ICPMS

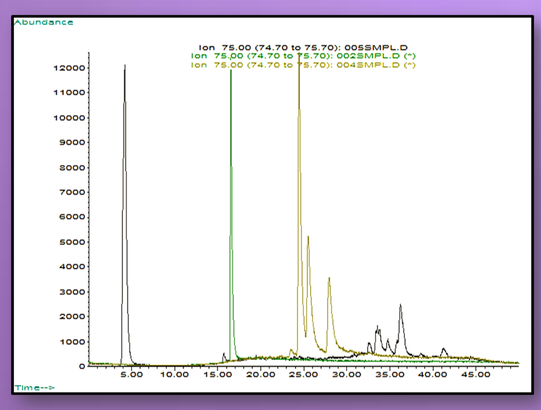

Figure 2: A comparison of the arsenolipid profile of a commercial oil, the cepalothorax oil and a standard of 100µg/L Ph_3AsO (black: cephalothorax oil; green: standard; yellow: commercial oil

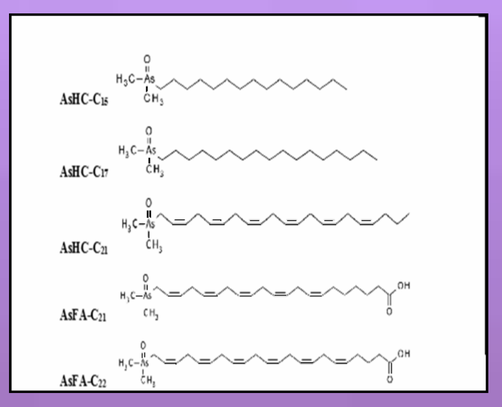

Figure 3: Chemical structures of arsenolipids of commercial oil; arsenic containing hydrocarbons (AsHCs) and arsenic-containing fatty acids (AsFAs) [3]

References: 1. Sele,V., Sloth, J. J. , Lundebye, A.K., Larsen, E. H., Berntssen, M. H. G.&Amlund, H.(2012). Arsenolipids in marine oil and fats: A review of occurrence, chemistry and future research needs. *Food Chemistry*,133, 618-630.
2. Bligh, E.G.&Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), 911-917.
3. Sele,V., Sloth, J. J. ,Holmelid, B.,Valdersnes, S., Skov, K.&Amlund, H. (2014). Arsenic-containing fatty acids and hydrocarbons in marine oils- determination using reversed-phase HPLC-ICP-MS and HPLC-qTOF-MS. *Talanta*, 121, 89-96.